

IN THE SPECIFICATION:

Please amend the paragraph beginning at page 1, line 9 as follows:

The present invention relates to a process for the preparation of protein hydrolysate from soy flour using a proteolytic enzyme of plant origin. Particularly, the present invention relates to a process for the preparation of protein hydrolysate from defatted soy flour using papain.

Please amend the paragraph beginning at page 1, line 15 as follows:

Presently about 6.8 M tons of soybean is produced in India and extracted for oil, ~~and the~~. The solvent extracted flour is exported to foreign countries for feed purposes. By providing additional facilities for the hygienic processing of soybean in the solvent extraction units, it is possible to obtain edible grade defatted flour having the desired functional characteristics. After the recovery of oil, 4.9 M tons of soy flour is available in India for utilization. A small portion of the total soybean produced also finds its use for different edible grade flours, protein isolate and texturized products ~~and the~~. The popularity of these products are greatly picking up globally increasing worldwide. Soybean – which contains about 40% protein – is an excellent source ~~of protein, which contains about 40% protein~~ this complex substance. New manufacturing techniques for high quality soybean foods have been developed by lowering or destroying ~~of the~~ anti-nutritional factors such as trypsin inhibitors.

Please amend the paragraph beginning at page 1, line 27 as follows:

United States Patent No. 5,180,597 ~~claimed~~ discloses a process for hydrolyzed vegetable protein with enhanced flavor, which contains no detectable level of monochlorodihydroxypropanol ~~is described~~. In the above reference, wheat gluten is ~~hydrolysed~~ hydrolyzed using Prozyme 6 (a fungal protease) at a temperature of 40-50°C, pH 6.5-7.0, enzyme concentration of 0.1-2% of substrate for a time period of 4h. The ~~hydrolysed~~ hydrolyzed protein is treated with gaseous HCl for deamidation before the addition of acid for inactivating the enzyme. The drawback in such hydrolysis is that ~~it~~ it is likely to lead to racemisation of amino acids and the addition of acid increases the salt content in the product.

Please amend the paragraph beginning at page 2, line 4 as follows:

United States Patent No. 5,077,062 ~~claimed~~ discloses a low sodium, low mono sodium glutamate soy hydrolysate that is prepared from soy material such as soy flour, sour meal or soy grits using fungal protease in water. The hydrolysis is conducted in the absence of acid or base at 90°C for 2 h. After deactivating the enzyme and de-watering the mixture, the resulting hydrolysate contains between 45 and 55 wt. % enzymatically hydrolysed soy based protein with an average molecular weight of 670,000 \pm 50,000. The fungal protease used is different from the enzyme used in the present invention. The process is energy intensive due to the high temperature (90°C) used.

Please amend the paragraph beginning at page 2, line 13 as follows:

United States Patent No. 4,757,007 ~~claimed a~~ discloses the preparation of two hydrolyzed products using a protease from soy protein. The soy protein is hydrolyzed with papain or pepsin after precipitating with alcohol. The drawback of the process is that it involves the separation of the mixture of hydrolyzed products. Hydrolysis is carried out using papain or pepsin. Acidification is carried out to bring down the pH to 2.5-5.0 to separate the two kinds of hydrolysates, which could lead to an increase in salt content.

Please amend the paragraph beginning at page 2, line 20 as follows:

European Patent No. 0148600 B-1 relates to the preparation of hydrolyzed protein from protein isolate after jet cooking or dynamic heating at 104°C for a few seconds and later ~~cooled~~ cooling in a vacuum chamber before performing hydrolysis using bromelin. The protein was precipitated at its isoelectric point from an aqueous extract of the material before the hydrolysis. The drawback of the process is that the starting material protein isolate ~~which~~ is more expensive. The process is a ~~multi-step~~ multi-step process, and is energy intensive. The process further needs machines like the jet cooker and a vacuum chamber.

Please amend the paragraph beginning at page 2, line 28 as follows:

European Patent No. 0223560 A2 ~~claimed~~ discloses a method describing the separation of protein hydrolysates with meat and cheese ~~flavor,~~ favor from proteinaceous feed stocks (e.g., containing soybean, gluten, whey, casein, hemoglobin, yeast, cereal or microbial proteins) by stepwise hydrolysis using an endopeptidase

followed by amino peptidase from *Streptococcus lactus*. The drawback of the process is that it is a multi-step process.

Please amend the paragraph beginning at page 3, line 4 as follows:

European Patent No. 0087246 B1 ~~claimed~~ discloses a process for the hydrolysis of soybeans, wheat gluten and cotton seeds using fungal protease from *Aspergillus* and pancreatin (trypsin, chymotrypsin A, B and C, elastase and carboxypeptidase A and B) ~~is described~~. Activated charcoal is used to treat the hydrolysate, which is used for nutritional improvement. The ~~draw-back~~ drawback of the process is that it involves more steps.

Please amend the paragraph beginning at page 3, line 10 as follows:

European Patent No. 0187048 A2 ~~described~~ describes the preparation of soy protein hydrolysate with 0.25 to 2.5% degree of hydrolysis (DH) using microbial rennet (*Mucor miehei*) and to be used as an egg white substitute. The enzyme used in the process is different and involves very low DH of soy protein.

Please amend the paragraph beginning at page 3, line 15 as follows:

United Kingdom Patent No. 2053228A ~~described~~ describes a process for the production of soy protein hydrolysate from partially defatted soy material by hydrolysis with proteolytic enzyme. The drawback of the process is that due to the partial defatting soy flour, ~~left-over~~ remaining oil comes in contact with the protein phase, which could lead to off-flavors.

Please amend the paragraph beginning at page 3, line 20 as follows:

United States Patent No. 4,324,805 ~~described~~ describes a method for producing soy protein hydrolysate and oil from partially defatted soy material by hydrolysis with a proteolytic enzyme. The soyflour is partially defatted by water washing at pH 3.5-4.5 and later ~~hydrolysed~~ hydrolyzed using water and a base to increase the pH. The DH is in the range of 8-12%. Oil is recovered from the wash water. Alcalase is the enzyme used. The drawback of the process is that it is a multi step process and due to partial defatting of soy flour, ~~left-over~~ remaining oil comes in contact with the protein phase which could lead to off-flavors. Enzyme inactivation is done by the addition of acid, which is likely to lead to ~~increase~~ increased salt content in the product.

Please amend the paragraph beginning at page 3, line 30 as follows:

United States Patent No. 3,640,725 ~~described~~ describes an enzymatic hydrolysis process for the production of soy protein hydrolysates. The soy seeds are comminuted and heated at 90-140°C. Protease (fungal and bacterial) is added at 25-75°C. The fiber is separated and slurry has ~~two phases-oil and~~ two phases – an oil phase and an aqueous phase. The aqueous ~~Aqueous~~ phase is brought to pH 4.5 to precipitate the protein, which is then concentrated. The starting material is not defatted and hence the residual oil could come in contact with the aqueous phase, which could lead to off-flavors.

Please amend the paragraph beginning at page 4, line 6 as follows:

Canadian Patent No. 905742 ~~described~~ describes a soy protein hydrolysate modified with pepsin to yield a product which, in the ~~in~~ presence of water and sugar, whips at a rapid rate to produce aerated products of low density.

Please amend the paragraph beginning at page 4, line 10 as follows:

European Patent No. 0797928 A1 ~~described~~ describes a process for the manufacture of a soy protein hydrolysate with a protease used selectively to decompose glycinin at a pH of 1.5-2.5. The pH used in the process is very low and therefore differs from the pH used in the present invention. Further, the aim of the process is to achieve a low glycinin content which is not the case in the present invention.

Please amend the paragraph beginning at page 4, line 16 as follows:

Reference may be made to a published paper entitled "Industrial production and application of soluble enzymatic hydrolysate of soy protein," (Olsen, H.S., Adler Nissen, J., ~~(1979), Process Biochemistry, 14(7), 6,8-10-11)~~ [1979], Process Biochemistry, 14[7], 6, 8, 10-11), wherein a method for preparation of soy protein hydrolysate from soy flakes washed at pH 4.5 followed by hydrolysis using alcalase is described. The solubility of the substrate is low at the acidic pH which is likely to result in low yields. The enzyme used is different from the enzyme used in the present invention.

Please amend the heading at page 4, line 24 as follows:

OBJECTS OF THE ~~Invention~~ INVENTION

Please amend the paragraph beginning at page 4, line 28 as follows:

Another object of the present invention is to provide a process for the preparation of protein hydrolysate with a specified degree of hydrolysis.

Please amend the paragraph beginning at page 4, line 31 as follows:

Still another object of the present invention is to provide a process for the preparation of protein hydrolysate soluble in water over a wide range of pH ~~value~~ values.

Please amend the paragraph beginning at page 5, line 6 as follows:

The invention provides a process for the preparation of protein hydrolysate from soy flour, ~~said~~ the process comprising the steps of: hydrolyzing an aqueous slurry of defatted soy flour containing 6-30% solid content w/v using proteolytic enzyme of plant origin at pH 5-9 and a temperature of $53\pm 5^{\circ}\text{C}$ under stirring for 30 min to 6 h; inactivating the enzyme by a ~~known~~ known manner; neutralizing the pH value of the slurry; separating the solids by a ~~known~~ known manner and drying the clarified liquor so obtained to get the said hydrolysate.

Please amend the paragraph beginning at page 5, line 14 as follows:

Accordingly, the present invention provides a process for the preparation of protein hydrolysate from soy flour, ~~said~~ the process comprising the steps of: hydrolyzing an aqueous slurry of defatted soy flour containing 6-30% solid content w/v

using proteolytic enzyme of plant origin at pH 5-9 and a temperature of $53\pm 5^{\circ}\text{C}$ under stirring for 30 min to 6 h; inactivating the enzyme by a known manner; neutralizing the pH value of the slurry; separating the solids by a known manner and drying the clarified liquor so obtained to get the said hydrolysate.

Please amend the paragraph beginning at page 5, line 24 as follows:

In another embodiment of the present invention, the plant origin proteolytic enzyme is selected from the group comprising of papain and bromelin.

Please amend the paragraph beginning at page 6, line 4 as follows:

In one ~~another~~ other embodiment of the present invention, the protein hydrolysate produced has decreased bitterness.

Please amend the paragraph beginning at page 6, line 16 as follows:

In one more embodiment of the present invention, high yield of protein hydrolysate with 30 to 35% degree of hydrolysis is obtained from the selected raw material taken.

Please amend the paragraph beginning at page 6, line 19 as follows:

In one ~~another~~ other embodiment of the present invention, a protein hydrolysate having creamy color and a yield of 20 to 25% (on flour basis) is obtained.

Please amend the paragraph beginning at page 6, line 22 as follows:

In ~~an~~ a further embodiment of the present invention, the protein hydrolysate has 3.0 to 5.0% moisture, 8.0 to 8.5% nitrogen and 30.0-35.0% degree of hydrolysis.

Please amend the paragraph beginning at page 8, line 4 as follows:

In still another embodiment of the present invention, the protein hydrolysate does not impart any undesirable flavor ~~for~~ to the finished product.

Please amend the line which appears on page 8, line 10, as follows:

The process involves the following process steps:

Please amend the paragraph beginning at page 8, line 12 as follows:

Soybean flour is derived from clean round beans. The cleaned beans pass through a cracking ~~process~~; process and the bean fragments are the graded on sieves and an aspirate system. The cleaned cracked meat is passed through a conditioner cooker and flaked. This is subjected to a solvent extraction process. The extracted flakes were desolventized and ground to 100 mesh. The specification for soy flour consists of (a) Moisture = 9% by mass (max); (b) Protein on dry basis = 48% by mass (min); (c) The total ash on dry basis = 7.2% by mass (max); (d) Acid insoluble ash on dry basis = 0.4% by mass (max); (e) Fat on dry basis = 1.5% by mass; (f) Crude fibre on dry basis = 4.2% by mass (max); (g) Aflatoxin = 30 ppb (max); (h) Residual solvent = 170 ppm; (i) Total bacterial count per gram = 50,000; (j) Coliform bacteria/g = 10; and (k) Salmonella bacterial – Nil.

Please amend the paragraph beginning at page 8, line 30 as follows:

Trinitrobenzenesulphonic acid (TNBS) procedure is an accurate, reproducible and generally applicable procedure for determining the degree of hydrolysis of food protein hydrolysates. The protein hydrolysate is dissolved/dispersed in hot 1% dodecyl sulphate to a concentration of $0.25 - 2.5 \times 10^{-3}$ amine equivalents/L. A sample solution (0.25 ml) is mixed with 2 ml of 0.2125 M sodium phosphate buffer (pH 8.2) and 2 ml of 0.1% Trinitrobenzenesulphonic acid, followed by incubation in the dark for 60 min at 50 C. The reaction is quenched by adding 4 ml. of 0.10 N hydrochloric acid (HCl) and the absorbance is read at 340nm. A 1.5mM L-leucine solution is used as the standard. Transformation of the measured leucine amino equivalents to a degree of hydrolysis is carried out by means of a standard curve for each particular protein substrate (Adler Nissen, J. (1979) [1979] J. Agri. Food Chem. 27, 6, 1256-1262).

Please amend the paragraph beginning at page 9, line 11 as follows:

Defatted soy bean flour was dispersed in water with a suitable solvent to solute ratio and the pH of the dispersion was adjusted using 6N sodium hydroxide or 6N hydrochloric acid. This was kept stirring for a few minutes with mechanical stirrer and the temperature raised to 50 - 55°C. At this stage 0.4-0.6 (w%) of papain on the basis of soy flour was added and stirring continued for 3-4 hours. At the end of the above time interval the temperature of the slurry was raised to 90-95°C for 5-10 minutes. The slurry was cooled to room temperature and the insoluble ~~carbohydrate-rich~~ carbohydrate-rich fraction in the dispersion was removed by centrifugation. The clarified protein hydrolysate was spray dried to obtain protein hydrolysate.

Please amend the paragraph beginning at page 10, line 11 as follows:

1 kg-g of defatted soy flour was dispersed in 5000 ml of water and the pH of the dispersion was adjusted to 5.0 using 1N HCl. The solution stirred with mechanical stirrer and then the temperature raised to 50°C. 5 g of papain was added and stirring continued for 4 hrs. The enzyme was inactivated by boiling for 5 min. The pH of the hydrolysate as adjusted to 6.5 using 6N NaOH. The slurry was cooled and centrifuged. The clear solution was spray dried. The yield was 20% (on flour basis) and degree of hydrolysis was 30%.

Please amend the paragraph beginning at page 10, line 19 as follows:

The particle size of the soy flour, ratio of enzyme to substrate, temperature, pH and time interval controls the end of enzymatic hydrolysis resulting ~~into minimizing~~ in minimal bitterness of the hydrolysate.

Please amend the paragraph beginning at page 10, line 23 as follows:

The soya protein hydrolysate obtained has a creamy colour and ~~an a~~ a yield of 20-25% (on the basis of the flour basis). the product has 3.0-5.0% moisture, 8.0-8.5% nitrogen and 30.0-35.0% degree of hydrolysis (TNBS procedure).

Please amend the paragraph beginning at page 10, line 27 as follows:

The soy protein hydrolysate obtained has 25-30 trypsin inhibitor Unit/mg (TIU/mg) activity, 95-98% nitrogen solubility index, 1.0-1.4% of salt content (measured as Cl ions) and 2 – 2.2 g/100 ml bitterness recognition threshold units. The

lipoxygenase and urease activities were not detectable. The amino acid composition of the soy protein hydrolysate obtain was similar to the amino acid ~~make-up~~ make-up of the starting raw material thereby retaining the nutritional value. The protein hydrolysate is less bitter compared to protein hydrolysate obtained from casein and is less hygroscopic in nature.

Please amend the paragraph beginning at page 11, line 5 as follows:

1. The enzyme employed is a food grade ~~commercially-available~~ commercially-available acceptable plant enzyme with broad specificity resulting in 30-35% degree of hydrolysis.
2. The product can be a good additive without imparting any undesirable flavour ~~for~~ to the finished product.
3. The hydrolysate ~~Hydrolysate~~ has a solubility which is independent of pH making it a suitable additive either in acid pH or alkaline pH.
4. The final product is in the dry form suitable for different food formulations and is easy to handle.

IN THE ABSTRACT:

Please amend the abstract of the disclosure as follows:

A process for preparation of protein hydrolysate from soy flour ~~said~~. The process ~~comprising~~ comprises the steps of hydrolyzing an aqueous slurry of defatted soy flour containing 6-30% solid content w/v using proteolytic enzyme of plant origin at ~~pH5-9~~ a pH of 5-9 and at a temperature of ~~53(5)C~~ 53±5°C under stirring for 30 minutes to 6

hours; inactivating the enzyme by a known manner; neutralizing the pH value of the slurry; separating the solids by a known manner and drying the clarified liquor so obtained to get the said hydrolysate.